Effect of vanillin on growth and enzyme production of *Lentinula edodes* at various stages during incubation

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Abstract : Effect of vanillin was examined on an edible mushroom, *Lentinula edodes*, at various stages during solid-state incubation. Addition of vanillin after 1 week incubation (at the growth stage) did not cause markedly effects. On the other hand, addition after 4 weeks incubation (at the stationary stage) enhanced mycelial growth, accelerated browning and induced fruiting body formation. The effect at the dormancy stage (after 6 months incubation) was observed remarkably on the enzyme production, while mycelium growth did not restart. Generally phenolic compounds can be an inducer of enzyme production and fruiting body formation but cannot be nutrient for mushrooms, so dormant mycelium did not grow in this study. This result suggests that phenolic compounds can act on dormant cell and would be able to activate dormant mycelium or late-growth strains by combination with suitable nutrients.

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Key words : laccase, *Lentinula edodes*, lignin, Mn-dependent peroxidase, vanillin.

Introduction

Lignin and lignin-related phenols were reported to affect on wood-rotting fungi in the aspect of mycelial growth (Ikegaya et al., 1993; Inaba et al., 1979; Kawamura et al, 1983; Shuen and Buswell, 1992), induction of fruiting body formation (Adachi et al., 2000; Ikegaya and Goto, 1988; Ikegaya et al., 1993; Kawamura et al., 1983; Yoshizawa et al., 2003), enzyme production (Faison and Kirk, 1985; Fåraeus, 1954; Haars and Hüttermann, 1983; Loberzewski and Trojanowski, 1979; Sethuraman et al., 1998) and so on. In our study of a white-rot fungus *Coriolus versicolor*, a phenolic compound vanillin directly activated phenol-oxidizing enzyme production and furthermore enhanced cellulolytic and xylanolytic enzyme productions in the co-presence of vanillin and oligo- or polysaccharides (Tsujiyama et al., 2000; Tsujiyama, 2003). This effect of phenol compounds is available for mushroom cultivation and solid-state fermentation by the enhancement of mushroom activity. Especially in mushroom cultivation, low cost management would be achieved with higher yields and shorter cultivation periods. However, activation mechanism to mushroom cell has not been clarified yet and it is unknown what action can occur on vegetative mycelium at any stage. In this study, vanillin was used as a model of lignin-related compounds, and its effect on an edible mushroom *Lentinula edodes* (Shiitake) was examined at various stages in the aspects of mycelial growth and enzyme production.

Materials and methods

Strain and incubation method

Test strain of *Lentinula edodes* (Berk.) Pegler KB2010 was provided from Kanebo Agritech Co. Ltd.

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Incubation was carried out on 15ml of peptone-glucose (PG) agar medium (Ikegaya et al., 1993) in a plant-incubation tube.

Addition of 1ml of vanillin solution (final concentration, 50μM) was performed after 1week, 4weeks and 6months. In the control only sterilized water was added.

Enzyme assays

Solid cultures were recovered and homogenized with 50mM sodium acetate buffer (pH 5.3). This suspension was used as crude enzyme solution. Laccase and Mn-dependent peroxidase were assayed as reported previously (Tsujiyama et al., 1992).

Weight of mycelium

After enzyme assays, crude enzyme solution was boiled and filtrated with pre-weighed filter paper. Filter paper was dried and weighed, and mycelium weight was calculated between the weights before and after filtration.

Results

Effect of the addition after 1 week incubation

To examine the effect on mycelium growth, three systems were performed; addition of vanillin solution, addition of sterilized water and no addition. Mycelium growths were regulated by additions of water or vanillin solution compared by no addition (Fig. 1). This was probably because water or vanillin solution covered culture surface to shut oxygen supply. On the other hand, enzyme production became markedly higher by addition of water or vanillin solution than no addition system (Fig. 2). This indicates that effect of vanillin was not determined in this stage, while covering culture surface with water, so called water-flooding treatment, induced phenol-oxidizing enzyme production.

Effect of the addition after 4 weeks incubation

Mycelial growth increased in both conditions with and without vanillin, and vanillin addition slightly enhanced mycelium weight more than the control system (Fig. 3). In the case of vanillin addition, the rate of browning was higher than that of the control system after 21 days incubation, and primordia formation was observed after 21 days

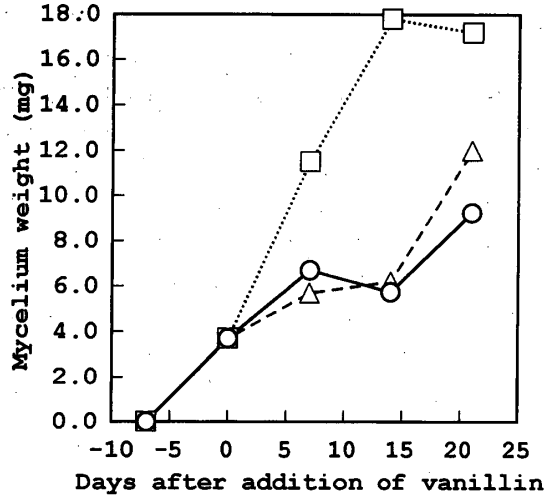

Figure 1 Effect of vanillin addition after 1 week incubation on mycelial growth of *Lentinula edodes*.
Symbols; *circles*: addition of vanillin solution, *triangles*: addition of distilled water, *squares*: no addition.

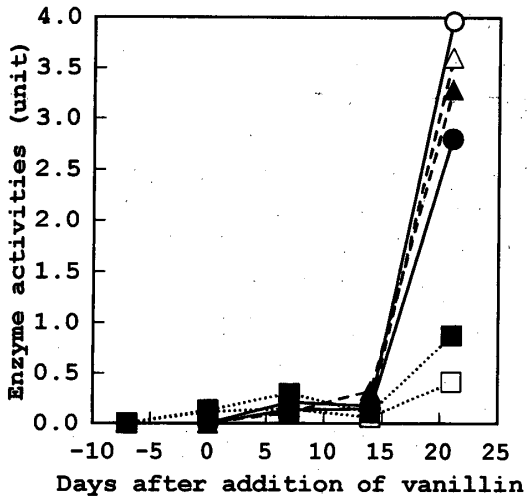

Figure 2 Effect of vanillin addition after 1week incubation on phenol-oxidizing enzyme productions of *Lentinula edodes*.
Symbols; *circles*: addition of vanillin solution, *triangles*: addition of distilled water, *squares*: no addition; *open*: laccase, *solid*: Mn-dependent peroxidase.

and fruiting body occurred at 28 days after vanillin addition. Even in the control system, small aggregates like immature fruiting body occurred at 35 days. In the late of incubation period, increase of mycelium weight was dependent on the fruiting body formation, so mycelium weight did not drastically increase in the tube where no fruiting body occurred. The rate of browning of mycelium by addition of vanillin was about two times of the control system. Note that it was not the exact value because the number of test tube had decreased in the late of incubation period.

Figure 4 shows enzyme production with addition of vanillin after 4 week incubation. Laccase and Mn-peroxidase activities were gradually decreased in the control system. However, in the case of vanillin addition, Mn-dependent peroxidase activity appeared to be induced and gradually decrease to the same level of the control. In the late of incubation period when fruiting body formation was found in some tubes, both enzyme activities roese in vanillin-addition systems. Ishikawa et al. (1983) reported that phenol-oxidizing enzyme activities decreased at the period of primordia occurrence and rose up after fruiting body formation. Ikegaya also pointed that phenol-oxidizing enzyme production decreased before fruiting body formation (Ikegaya et al, 1993). In this study, decrease of enzyme activities during 7-21days after vanillin addition also would relate to the formation of fruiting body. Therefore, the enzyme production of this strain could not be regulated only by water addition without low-temperature treatment, and so fruiting body formation did not occur. However, the enzyme solution prepared from the tube where fruiting body formation occurred did not indicate marked high enzyme activities.

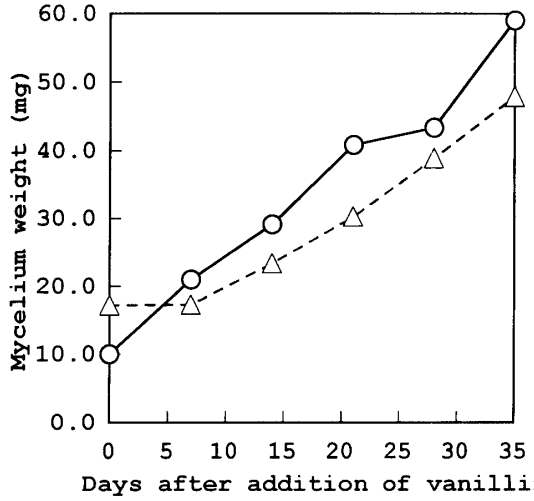

Figure 3 Effect of vanillin addition after 4 weeks incubation on mycelial growth of *Lentinula edodes*.
Symbols; *circles*: addition of vanillin solution, *triangles*: addition of distilled water.

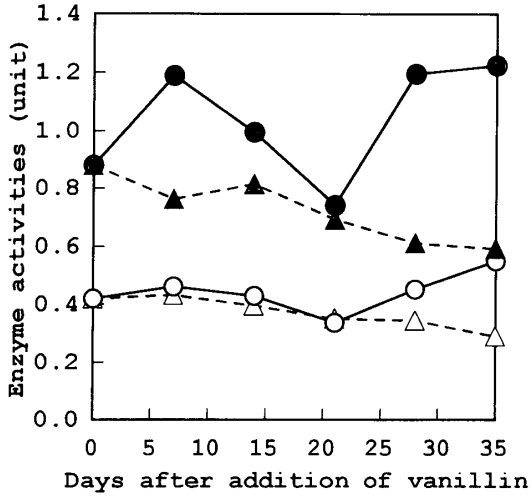

Figure 4 Effect of vanillin addition after 4 weeks incubation on phenol-oxidizing enzyme productions of *Lentinula edodes*.
Symbols; *circles*: addition of vanillin solution, *triangles*: addition of distilled water; *open*: laccase, *solid*: Mn-dependent peroxidase.

Effect of the addition after 6 months incubation

Effect of vanillin on mycelial growth was not determined after 6 months incubation (Fig. 5). However, laccase and Mn-peroxidase were produced at higher level by vanillin addition than that of the control system (Fig. 6). Culture after 6 months, incubation was slightly dried and mycelium seems to be dormant. Addition of water could release dormancy and furthermore mycelium was activated by stimulation of vanillin. However, enzyme production did not continue, as mycelium growth did not restart.

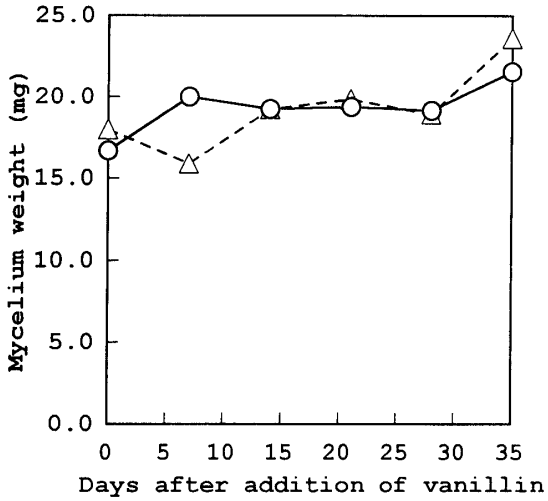

Figure 5 Effect of vanillin addition after 6 months incubation on mycelial growth of *Lentinula edodes*.
Symbols; *circles*: addition of vanillin solution, *triangles*: addition of distilled water.

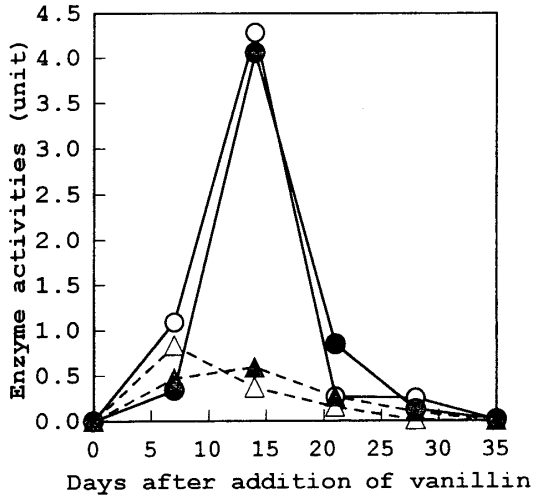

Figure 6 Effect of vanillin addition after 6 months incubation on phenol-oxidizing enzyme productions of *Lentinula edodes*.
Symbols; *circles*: addition of vanillin solution, *triangles*: addition of distilled water; *open*: laccase, *solid*: Mn-dependent peroxidase.

Discussion

In the purpose for the examination of the effect of phenolics on *Lentinula edodes*, mycelium growth and phenol-oxidizing enzyme production were examined by addition of vanillin at three stages; the growth stage (after 1 week incubation), the stationary stage (after 4 weeks incubation) and the dormant stage (after 6 months incubation). In this study, enzyme assay was carried out as an indicator of the response to phenolic compounds, not as the estimation of ligninolytic ability; induction of phenol-oxidizing enzyme was related to the recognition at the cell level.

In the growth stage, effect of vanillin addition was observed in neither mycelium growth nor enzyme production. Vanillin addition after 4 weeks (at the stationary stage) accelerated mycelium growth and browning and induced fruiting body formation. In the case of dormant mycelium that seemed to be dead, addition of vanillin caused the expression of enzyme production but did not start mycelium growth. This observation was not reported previously. The reason that growth did not restart was probably because the nutrient in culture has been lost or unnecessary metabolites have accumulated.

Previously, lignin-related compounds were reported to cause enzyme production, enhancement of mycelium growth and fruiting body formation. In this study, vanillin played these roles at three tested stages, mainly at the stationary stage. Thus, the screening of the phenolic compound, which can act physiologically to mushroom more than vanillin, would be useful for cultivation purposes. Note that, as phenolic compounds generally could not be nutrient for mushroom, combination with the suitable nutrients would cause the activation of dormant mycelium and late-growth strains.

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様々な生育段階におけるシイタケの生長および酵素生産に対するバニリンの効果

辻 山 彰 一

摘要：固体培地の様々な成育段階における食用きのこシイタケに対するバニリンの効果について調べた。成長段階である培養開始1週間後にバニリンを添加したところ明確な作用は確認できなかった。定常段階である4週間後に添加を行ったところ，菌糸生長は増進され，菌糸の褐変化の促進および子実体誘導が起こった。培養開始6ヵ月後の休眠状態の菌糸においては，添加により菌糸成長の再開は起こらなかったがフェノール酸化酵素の生産が顕著に認められた。一般にフェノール化合物は酵素生産および子実体形成の誘導物質として機能するが，栄養成分とはならないため，本研究でも休眠菌糸は生長しなかった。以上の結果，フェノール化合物は休眠細胞に作用することがわかり，適切な栄養成分との組み合わせにより休眠菌糸や生長不良菌株の活性化作用を持ちうることが示唆された。